



ICM-MS phenotyping in applied animal sciences: Two examples

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ICM-MS phenotyping in applied animal sciences: Two examples

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- Basic and applied animal sciences
- Reproduction and Behaviour
- Infectiology and Public Health
- Avian Research Unit



Intact Cells MALDI-TOF MS

- Identification of cell-specific peptide/protein MS fingerprints
- “Intact cell” means that whole cells are subjected directly to MS analysis without any preparatory steps
- Widely used in microbiology for biotyping
- Starting to be used in pathology and diagnostics

1

Phenotyping
chicken male
fertility



Why phenotyping male fertility?

Sperm quality tests evaluate **individual sperm cells functions**:
motility, integrity, acrosome reaction, etc.

Often **poorly correlated** with *real* fertility

A **new paradigm of sperm quality testing** is needed: global evaluation
of sperm functions

Background: Pilot study by Labas et al., 2014:

-Intact cell MALDI-TOF mass spectrometry (**ICM-MS**) = fast, reliable and relatively inexpensive tool to phenotype male fertility



molecular basis of fertility (top-down HRMS)

OBJECTIVES:



- 1- Acquisition of sperm cells ICM-MS profiles in a standardized and automated way.
- 2- Evaluation of the diagnostic performance of ICM-MS profiles comparison to predict fertility.
- 3- Identification of the endogenous peptides/proteins represented in the sperm cells ICM-MS spectra, particularly those linked with fertility.

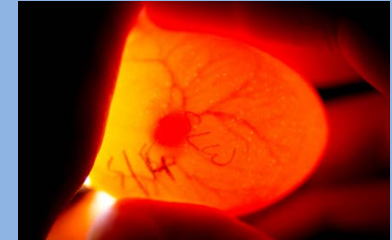
Animal model and reference fertility rates

Our model is.....the chicken!!!



72 roosters of 2 divergent pure genetic lines: meat line (n=36) and a egg laying line (n=36).

Fertility rates (% fertile/incubated eggs) : artificial insemination 10 females/male.



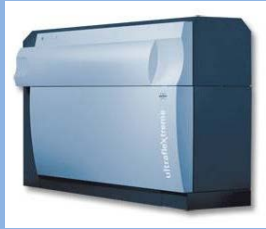
Fertility rates
cut-off values



Intact Cells MALDI-TOF MS

10^6 of fresh, washed (Tris-Sucrose) sperm cells + Matrix (20mg/mL Sinapinic acid, 2% TFA, 50% ACN)

12 spots / sample, 3 consecutive runs ---- 36 technical replicates



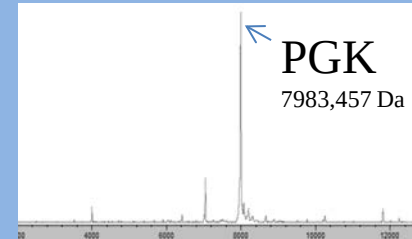
3 ejaculates per male (high day-to-day variability)

Spectra automatic acquisition in a Bruker UltrafleXtreme MALDI-TOF instrument (mass range 1-20 kDa).



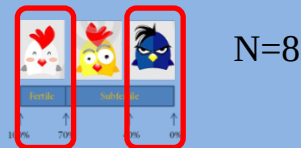
External calibration : Peptide/protein mix calibrant

Internal calibration: Lock-mass using peak 7983,457 Da



Pre-processing: ClinProTools 3.0 (Bruker)

Model construction



Quick Classifier
Supervised Neural Network
Genetic Analyser

Validation



Classification as Fertile
or Subfertile by >50%
spectra from each male

*Fertility-
predictive models
construction
using
ClinProTools 3.0*

Evaluation

Recognition capability: Ability
of each model to correctly
identify its component spectra

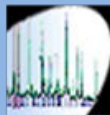
Cross-validation: Ability to
handle variability among spectra

Diagnostic Accuracy

Sensitivity / Specificity

Positive and negative
predictive values

Positive and negative
likelihood ratios



In-vitro sperm quality tests

- Viability
- Subjective mass motility evaluation
- Objective motility evaluation measured with computer-assisted sperm analysis (CASA) system with an HTM-IVOS

3 ejaculates per male

Diagnostic accuracy

Sensitivity / Specificity

Positive and negative predictive values

Positive and negative likelihood ratios

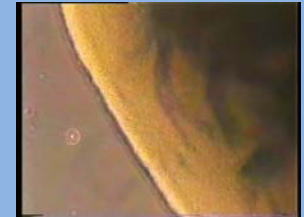


Viable

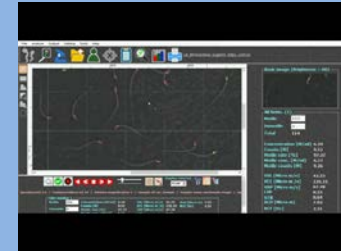
Not viable



Mass motility = 1/5



Mass motility = 5/5



CASA System

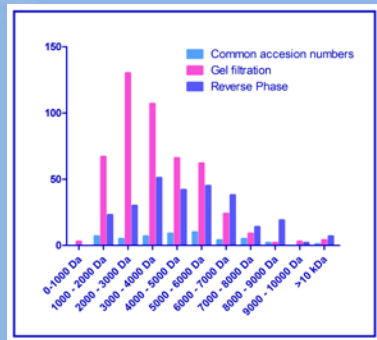
Sperm cells
protein extracts
Tris-Urea 6M
Sonication

Fractionation



Reverse Phase Chromatography

XBridge BEH C18, Waters



Gel Filtration Chromatography

Superdex 75 10/300 GL, GE Healthcare

μLC-MS/MS:



Monolithic PS-DVB
PepSwift, Dionex

LTQ Orbitrap Velos,
Thermo Scientific

Target resolution 100000

Range 400-2000 m/z

Isolation: top 10 most intense ions with charge states ≥ 2

Fragmentation/ HCD 38% Collision energy
Ion selection thr: 500 counts; isol: m/z 3

Identification and structural characterization:
 Search 1: Monoisotopic mass, 10ppm tolerance
 Search 2: Average mass, 3 kDa tolerance
 Search 3: Average mass, 25k Da tolerance
 $p\text{-score} \leq 1 \times 10^{-5}$



The screenshot shows the PDB search results for the entry 2G9L. The top section displays the search criteria: Search Name (CPDsearch/Pepcidate/Act), Search Type (Structure), Biomarker Search (No), Database Description (Galus galus_012_06 Top Down), Precursor Mass Type (Monoisotopic), Precursor Tolerance (5 ppm), Fragment Mass Type (Monoisotopic), Fragment Tolerance (10 ppm), Δm Mode (Delta), Deuterie (unchecked), Hit Filtering (checked), Min # of Matching Fragments (5), Min % of Matching Fragments (0), Min Score (empty field), Max Proteins to Return (10), and Food Modifications (Cysteine, Methionine, Lysine, Histidine, Leucine). The bottom section shows PTM Handling options: All PTMs (selected) and Only PTMs (unselected).

Search Criteria	Value
Search Name	CPDsearch/Pepcidate/Act
Search Type	Structure
Biomarker Search	No
Database Description	Galus galus_012_06 Top Down
Precursor Mass Type	Monoisotopic
Precursor Tolerance	5 ppm
Fragment Mass Type	Monoisotopic
Fragment Tolerance	10 ppm
Δm Mode	Delta
Deuterie	☐
Hit Filtering	☒ Include Modified Forms
Min # of Matching Fragments	5
Min % of Matching Fragments	0
Min Score	
Max Proteins to Return	10
Food Modifications	Cysteine, Methionine, Lysine, Histidine, Leucine
PTM Handling	All PTMs (selected)

ICM-MS- based models vs. Traditional tests

85,5 % of shared m/z masses but differences in **TIC** values : no common model



Meat line (GA)

Recognition Capability	Cross Validation
99.4 %	93.48 %

Parameter	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
ICM-MS	84.6	55.0	55.0	84.6	1.9	0.3
Mass motility	69.2	55.0	50.0	73.3	1.5	0.6
Viability	76.9	35.0	43.5	70.0	1.2	0.7
% Motiles	61.5	40.0	40.0	61.5	1.0	1.0

Laying line (SNN)

Recognition Capability	Cross Validation
92.28 %	80.3 %

Parameter	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
ICM-MS	100.0	75.0	53.3	100.0	4.0	0.0
Mass motility	71.4	78.6	45.5	91.7	3.3	0.4
Viability	71.4	57.1	29.4	88.9	1.7	0.5
% Motiles	71.4	78.6	45.5	91.7	3.3	0.4

ICM-MS: Global analysis of sperm cells functions

GO - Biological Process

Respiratory Electron Transport Chain

Cellular Metabolic Process

Small Molecule Metabolic Process

Protein Polymerization

Microtubule Cytoskeleton Organization

Microtubule-based Process

GO - Molecular Function

Structural Constituent of Cytoskeleton

ATPase Activity

Proton-transporting ATP Synthase Activity, Rotational Mechanism

Transmembrane Transporter Activity

Creatine Kinase Activity

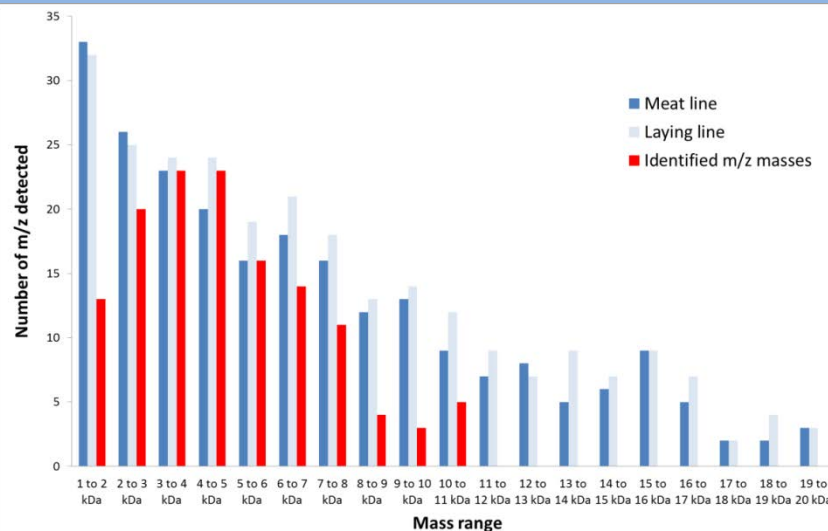
MHC Class II Protein Complex Binding

Protein Binding Involved in Protein Folding

ATP Binding

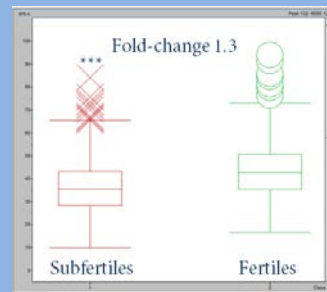
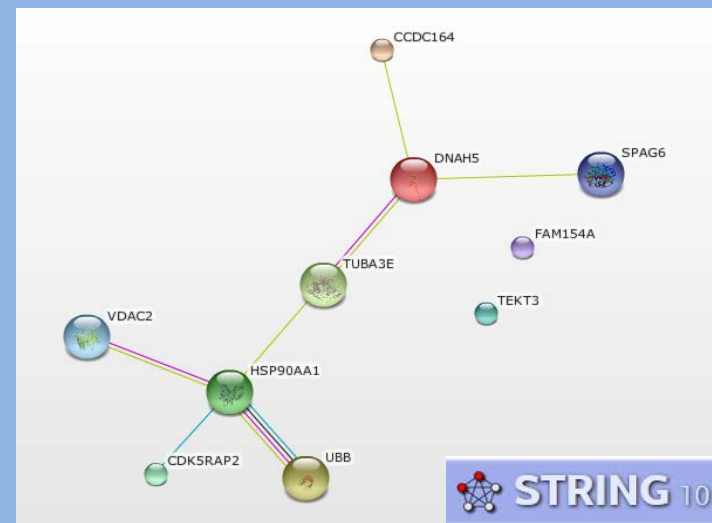
Proton-transporting ATPase Activity, Rotational Mechanism

119/256
m/z
masses
identified



Masses employed to generate models 12/27

Mass (kDa)	Gene Name	Ratio SubF/F	Protein name	Role associated with sperm cells functionality
7035,24	LRD	1,8	Left-right dynein protein	Flagellar motility
6539,45	TUBA3E	1,7	Tubulin, Alpha 3e	Cell integrity and Flagellar motility
5226,48	CDK5RAP2	1,6	CDK5 regulatory subunit associated protein 2	Centrosome organization during fertilization
6695,03	HSP90AA	1,3	Heat shock protein 90 alpha	Chaperone, stabilisation of key proteins
2574,18	TEKT3	1,2	Tektin 3	Flagellar motility
8569,95	UBB	1,2	Ubiquitin	Protein degradation
4538,03	SPAG6	1,1	Sperm associated antigen 6	Cell integrity and Flagellar motility
4010,91	FAM154A	1,05	Family with sequence similarity 154, member A	Flagellar motility
6615,77	VDAC2	0,9	Voltage-dependent anion channel	Motility/capacitation/fertilization
4777,2	DRC1	0,9	Dynein Regulatory Complex Subunit 1 Homolog	Flagellar motility
2400,65	FER1L4	0,8	Fer-1-like protein 4 / Otoferlin	Membrane traffic/Sperm activation
2187,08	LV1L2	0,8	Ig lambda chain V-1 region-like	Motility



Immunoblotting



Subfertiles Fertiles

Fold-change 1.6

Conclusions

Fertility-predictive models can be generated from sperm cells ICM-MS spectra with high diagnostic performance

Allows for global testing of different sperm cell functions

Interpretation of molecular basis of fertility





2

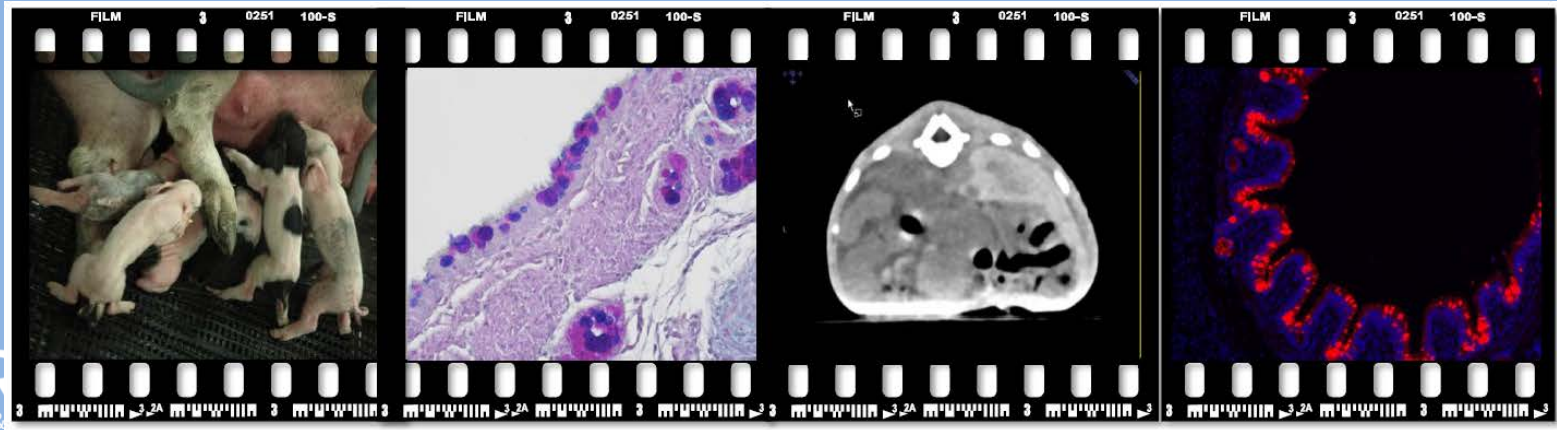
Phenotyping of
Swine CFTR^{+/+}
vs. CFTR^{-/-}
neutrophils

Example 2: Swine Cystic Fibrosis-mutant neutrophils phenotyping (*Ignacio Caballero-Posadas*)

Caused by the presence of mutations in both copies of the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) protein.

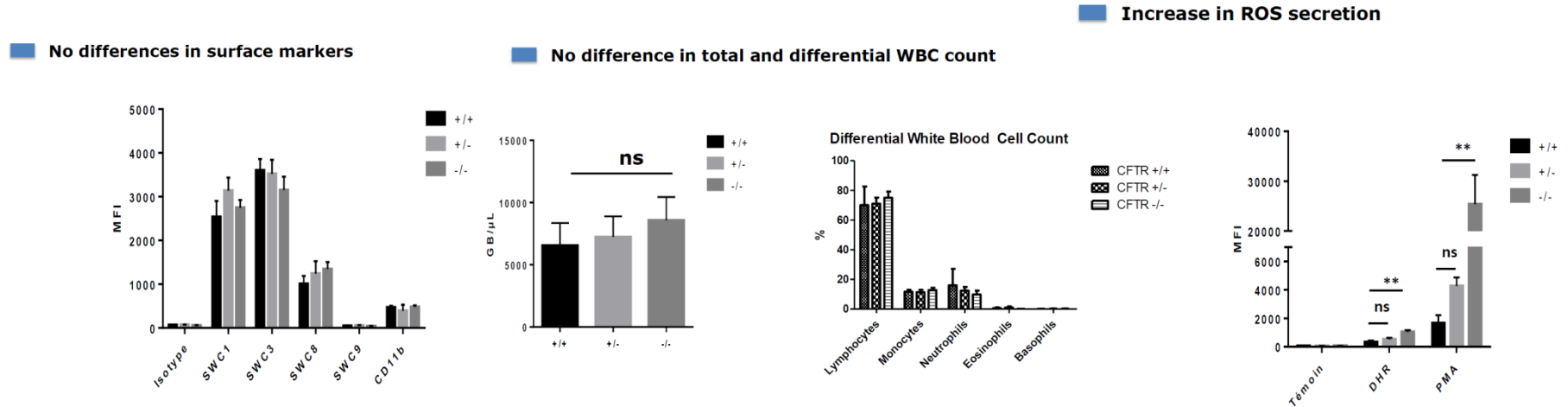
Affects mostly the lungs but also the pancreas, liver, kidneys, and intestine.

Pig transgenic model CFTR^{-/-}



Altered neutrophil function?

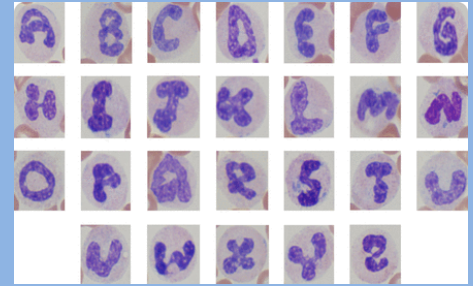
- Presence of CFTR → Phagolysosome (Painter et al. 2006, 2008, 2010)
- Defective killing (chlorination phagolysosome)
- Altered chemotaxis
- Increased oxidative burst
- Increased protease release



5×10^5 cells/spot

7 CFTR^{+/+} and 7 CFTR^{-/-}

8 technical replicates

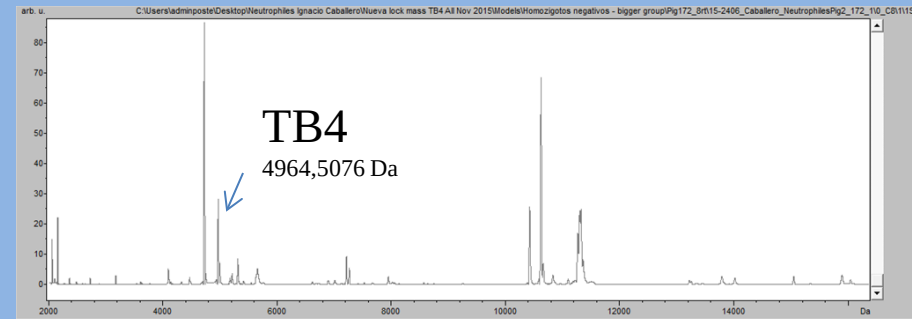


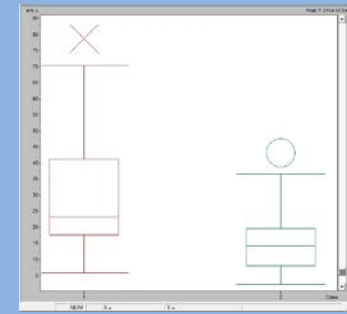
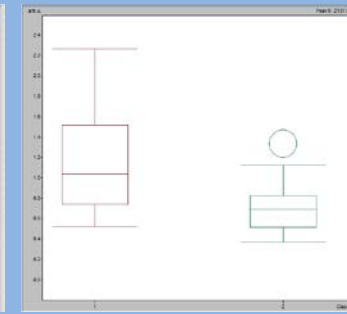
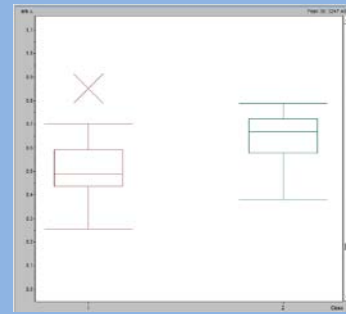
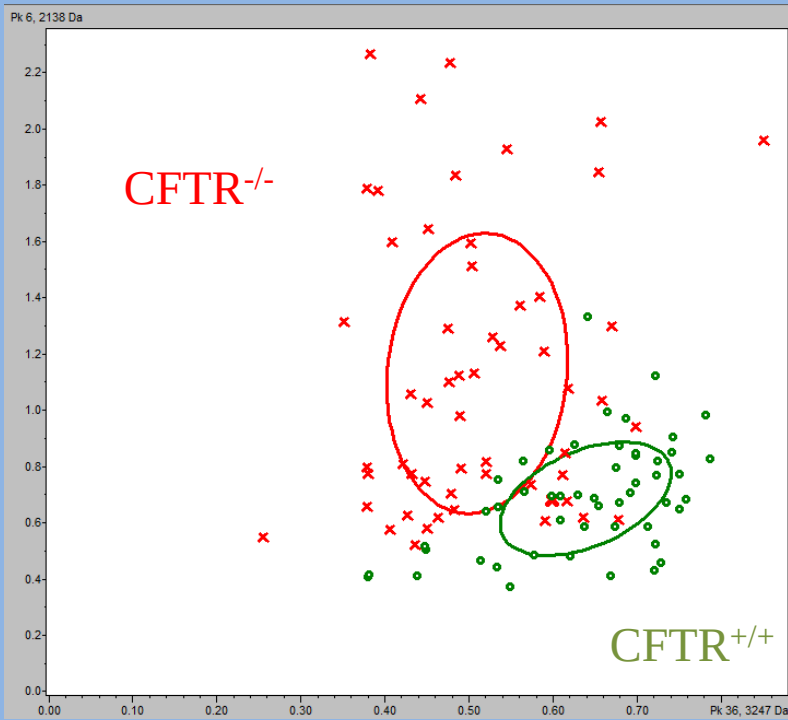
Spectra manual acquisition in a Bruker UltrafleXtreme MALDI-TOF instrument (mass range 1-20 kDa).

External calibration : Peptide/protein mix calibrant

Internal calibration: Lock-mass using peak 4964,5076 Da (TB4)

Pre-processing: ClinProTools 3.0 (Bruker)

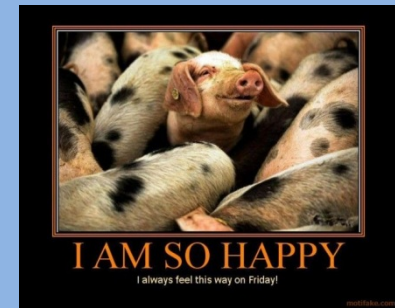
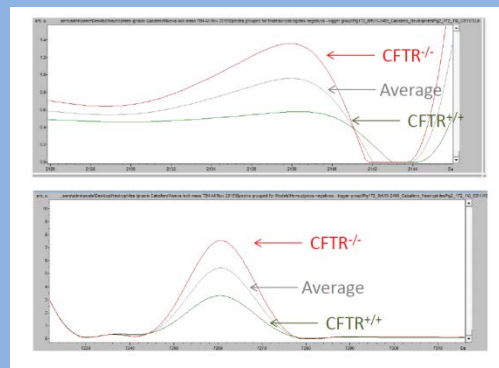




Index	Mass	DAve	PTTA
36	3247.48	0.14	< 0.000001
6	2137.67	0.5	0.0000036
7	2154.58	17.17	0.00000367
114	7260.62	4.2	0.00000367
28	2980.76	0.13	0.00000367

Genetic Analyser

Recognition Capability	Cross Validation
100 %	92.73 %





Acknowledgements_

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